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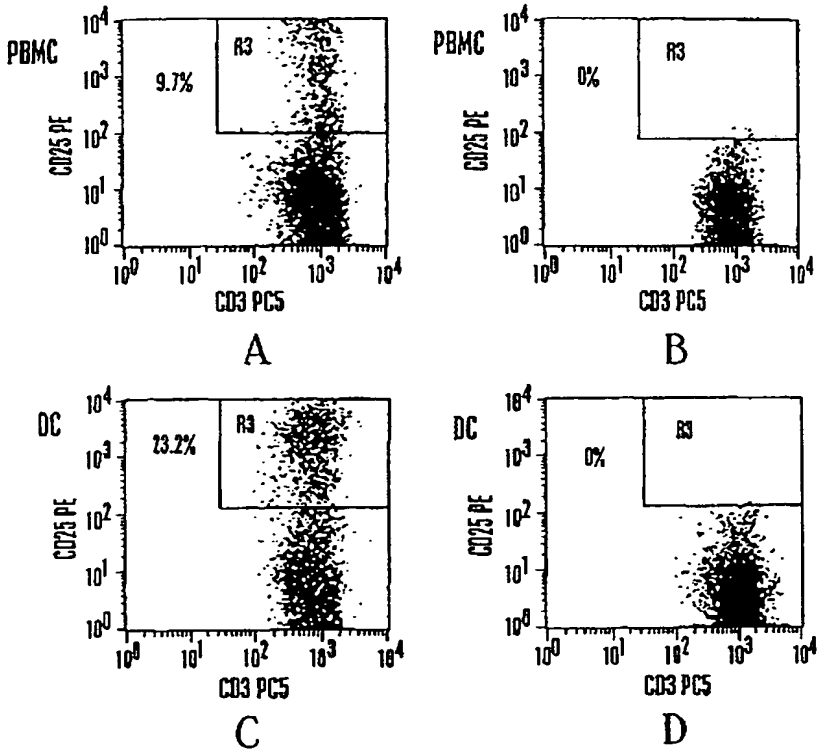
(54) Title: METHODS FOR DEPLETING AND ISOLATING ALLOREACTIVE AND ANTIGEN-REACTIVE T CELLS FROM HEMATOPOIETIC DONOR CELLS



(57) Abstract: The invention provides methods for depleting alloreactive or antigen-reactive T cells from a hematopoietic donor cell population, by co-culturing a recipient-derived dendritic cell population or cultured macrophage population with the donor cell population, under conditions wherein the recipient-derived population activates donor T cells in the donor cell population, and killing or removing the activated T cells. Also provided are hematopoietic donor cell population depleted of, or enriched for, alloreactive or antigen-reactive T cells prepared by these methods. The invention further provides methods of using the depleted and isolated hematopoietic donor cell population in transplant procedures.

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METHODS FOR DEPLETING AND ISOLATING ALLOREACTIVE AND ANTIGEN-REACTIVE T CELLS FROM HEMATOPOIETIC DONOR CELLS

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The invention relates to the field of medicine and, more specifically, to improved methods for depleting and isolating reactive T cells from hematopoietic cells for human transplantation.

BACKGROUND INFORMATION

Hematopoietic stem cell transplants, which are transplants of blood cells or bone marrow from the same individual (an autologous transplant, or autograft) or different individual (an allogeneic transplant, or allograft), are of proven benefit in treating a variety of immune dysfunctions and malignancies. However, the widespread application of allogeneic transplant procedures is restricted by the availability of suitable donors.

A suitable allogeneic donor is an individual with an identical or near identical profile of cell-surface antigens known as major histocompatibility antigens (MHC) or HLA antigens. There are many alternative forms (alleles) of each of the HLA antigens, and thus the chance of two unrelated individuals being closely HLA-matched is extremely small.

As HLA antigen loci are closely linked, an individual inherits the HLA alleles as two sets, one from each parent. Therefore, a certain percentage of

individuals eligible for an allogeneic stem cell transplant will have an HLA-identical relative. Nearly all eligible patients will have a relative who is haploidentical, sharing half of the HLA antigens with the patient.

One of the major barriers to successful transplantation between HLA-identical or haploidentical individuals is the risk of developing potentially life-threatening graft-versus-host disease (GVHD). GVHD occurs when donor T cells recognize the tissues of the recipient as foreign and attack them, causing a severe inflammatory disease. The cost of treatment for GVHD in the United States is estimated to be $80,000 per patient.

The activity of donor T cells against HLA antigens, and other minor histocompatibility antigens, on the surface of recipient cells is at the center of GVHD pathology, and approaches to date to control GVHD have employed *in vivo* or *ex vivo* methods that suppress the function of T cells. The majority of efforts have focused on removing or suppressing the activity of the entire repertoire of donor T cells, or major subsets of these, resulting in global immunosuppression in the recipient. However, donor T cells, while contributing to the development of GVHD, also contribute positively to transplant outcome by facilitating engraftment and controlling opportunistic infections. Donor T cells also are associated with a beneficial "graft-versus-tumor" effect. Thus, indiscriminate depletion of T cells from the allograft, while reducing GVHD, also increases graft failure, susceptibility to opportunistic infections, and leukemia relapse rate.

Only a very small portion of a donor's T cell repertoire (1:100 to 1:1000 cells) is alloreactive and responsible for generation of GVHD. For this reason, a strategy to selectively eliminate or inactivate only the alloreactive cells, without affecting the entire T cell repertoire, would significantly improve the safety of allotransplantation by reducing infectious complications while preventing GVHD.

One approach that has been used to selectively inactivate alloreactive T cells is to kill or physically remove them. Reactive donor T cells, when cultured with irradiated recipient cells, express activation antigens. The activated T cells can then be physically removed by fluorescence-activated cell sorting or immunomagnetic cell separation using specific antibodies that identify these activation antigens, or killed using toxin-conjugated antibodies.

T cell activation is controlled by antigen presenting cells (APC) that include macrophages, B cells, and dendritic cells. APC not only present the MHC-peptide complex to engage T cell receptors, but also deliver costimulatory signals that are necessary for T cell activation. Studies focused on activating and removing or killing alloreactive T cells have used the recipient's bulk peripheral blood mononuclear cells (PBMCs) as stimulators to activate donor alloreactive T cells (see, for example, Koh et al., Bone Marrow Transplantation 23:1071-1079 (1999); Mavroudis et al., Bone Marrow Transplantation 17:793-799 (1996); Garderet et al., Transplantation 67:124-130 (1999); Rencher et al., Bone Marrow Transplantation 18:415-420 (1996); and Montagna et al., Blood 93:3550-3557 (1999)).

However, it is not known whether allostimulation by PBMCs is sufficient to stimulate all alloreactive T cells to express activation markers, particulary in instances where the donor and recipient are closely or identically HLA-matched. Accordingly, allostimulation by PBMCs may not be sufficient to allow the effective depletion of GVHD-causing T cells.

Therefore, there exists a need to develop improved methods of activating alloreactive T cells in a donor graft. Such methods would allow alloreactive T cells to be effectively removed from a donor graft, and thus reduce the risk of GVHD upon introduction of the donor graft into the patient.

Allostimulation by bulk PBMCs is also expected to stimulate T cells that are reactive with antigens present on the surface of the various different types of hematopoietic cells present in a PBMC preparation. Therefore, stimulation by bulk PBMCs, followed by depletion of activated donor T cells, is expected to non-specifically deplete those donor T cells that are reactive with recipient hematopoietic cells, including T cells reactive with malignant hematopoietic cells. T cells reactive with hematopoietic cells are of particular benefit in "mini-transplant" procedures, in which the recipient's immune system is only partially ablated by low-dose radiation or chemotherapy, and the donor graft is introduced to attack the remaining hematopoietic cells. T cells reactive with recipient malignant cells are of benefit in eliciting a graft-versus-leukemia reaction in the therapy of leukemia.

Therefore, there also exists a need to develop more selective methods of depleting alloreactive T cells from a donor allograft, so that beneficial T cells can be

retained in the graft, if desired. A donor graft produced by such a method would have a selective ability to ablate the host immune system in mini-transplant procedures, and/or a selective ability to elicit a graft-versus-leukemia response, with a reduced potential for inducing GVHD.

Over the last few years, autologous stem cell transplants have been used in the treatment of patients with severe autoimmune diseases, and allogeneic stem cell transplants have been tested in animal models (see, for example, Tyndall et al., Bone Marrow Transplantation 24:729-734 (1999); and Fassas et al., J. Clin. Immunol. 20:24-30 (2000)). Many autoimmune diseases result from host T cells recognizing certain of the host's own tissues as foreign and attacking them. In stem cell transplant procedures to date for treating autoimmune diseases, the patient's own mobilized blood or marrow cells are removed, then residual immune cells in the patient are eradicated, and all or some T cell depleted subset of the cells (e.g. CD34+ cells) are reinfused. However, as with non-selective depletion of T cells in an allograft, non-selective depletion of T cells from an autograft can prevent the patient from successfully responding to opportunistic infections. On the other hand, if the patient cells are reinfused without T cell depletion, some autoreactive T cells will also likely be reinfused, reducing the efficacy of the procedure.

Therefore, there exists a need to develop selective methods of depleting autoreactive T cells from an autograft. Such methods would allow autoantigen-reactive T cells to be effectively removed from an autograft, and thus improve the efficacy of autografts for the treatment of autoimmune diseases.

The present invention satisfies the needs described above, and provides related advantages as well.

SUMMARY OF THE INVENTION

The invention provides methods for depleting alloreactive or antigen-reactive T cells from a hematopoietic donor cell population, by co-culturing a recipient-derived dendritic cell population or cultured macrophage population with the donor cell population, under conditions wherein the recipient-derived population activates donor T cells in the donor cell population, and killing or removing the activated T cells. Also provided are hematopoietic donor cell populations depleted of, or enriched for, alloreactive or antigen-reactive T cells prepared by these methods. The invention further provides methods of using the depleted and isolated hematopoietic donor cell populations in transplant procedures, such as to reduce the risk of developing graft-versus host disease, to ablate a recipient's immune system, to induce a graft-versus-leukemic cell reaction, to treat cancer and infectious disorders, or to treat an autoimmune disease.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the scatter profile (A), and the expression of indicated phenotypic markers (B-F), of dendritic cells differentiated from monocytes in culture. The dotted line represents the isotype control; the solid line represents specific staining. The X-axis represents fluorescence intensity; the Y-axis represents relative cell numbers.

Figure 2 shows expression of the indicated surface activation markers on donor T cells in response to coculturing with recipient-derived peripheral blood mononuclear cells (PBMC)(A-C) or dendritic cells (DC) (D-F).

Figure 3 shows CD25 expression by donor T cells cocultured with recipient-derived PBMC (A and B) or dendritic cells (C and D), before (A and C) or after (B and D) immunomagnetic depletion with anti-CD25 antibodies.

Figure 4 shows CD25 expression upon restimulation with recipient DCs, by donor T cells which were originally stimulated with recipient PBMC (A and C) or originally stimulated with recipient DCs (B and D). A and B show CD25 expression before, C and D are after, immunomagnetic depletion with anti-CD25 antibodies.

Figure 5 shows the relative proliferation of donor T cells stimulated by recipient-derived dendritic cells (filled squares), cultured macrophages (open triangles) or uncultured monocytes (filled circles). The X-axis represents the number of recipient-derived stimulator cells (RDSC) to 1 x 10^5 T cells used in the coculture; the Y-axis represents incorporation of ^3H-thymidine into DNA, in counts per minute (cpm).

DETAILED DESCRIPTION OF THE INVENTION

The invention provides methods for depleting or isolating reactive T cells, including alloreactive and antigen-reactive T cells, from hematopoietic donor cell populations. Methods currently used in the art for depleting alloreactive T cells from donor populations generally use recipient-derived peripheral blood

mononuclear cells (PBMCs) as stimulators. As disclosed herein, dendritic cells (DCs) and activated macrophages (MAKs) are superior to PBMCs for this purpose, in that they more effectively and more selectively stimulate reactive T cells. Therefore, the invention methods can be used to more effectively deplete hematopoietic donor cell populations of T cells with undesired reactivities, while retaining or selectively isolating T cells with desired reactivities.

As used herein, the term "hematopoietic donor cell population" refers to any population of cells derived from a hematopoietic tissue that is removed from a mammalian individual (a "donor") and is suitable to be reintroduced, in whole or in part, into the same ("autologous") or different ("allogeneic") mammalian individual (a "recipient"). The hematopoietic donor cell population can be either freshly obtained, cultured or frozen, but has been maintained under conditions suitable to maintain sterility and promote viability.

Preferably, the mammalian individuals are humans, but for certain applications can also be veterinary animals or research animals. For example, in preclinical studies, the methods of the invention can be practiced with animals that serve as credible models of human disease, such as non-human primates, pigs, dogs, cats and rodents. Those skilled in the art understand which animals serve as appropriate models for a human disease of interest.

A hematopoietic donor cell population is a cell suspension derived from a hematopoietic tissue, such as peripheral blood, umbilical cord blood, or bone marrow. In one embodiment, the hematopoietic donor cell population comprises a "leukocyte population" derived

from a hematopoietic tissue. In other embodiments, the leukocyte population is a blood or bone marrow population enriched for mononuclear cells, enriched for lymphocytes, enriched for T lymphocytes or enriched for a desired subset of T lymphocytes, as described further below.

Throughout this disclosure the terms "X cell population," "population of X cells," "population enriched for X cells," where X is an indicated subset of hematopoietic cells, are used interchangeably, and are intended to mean that the population has been enriched so as to contain a higher percentage, as further specified herein, of the indicated cells than is found in normal adult blood or marrow.

The cellular composition of normal adult human blood is about 95% red blood cells, about 5% platelets, and about 0.1% leukocytes. Leukocytes are composed of about 30-40% mononuclear cells (including lymphocytes, monocytes, stem and progenitor cells, and circulating dendritic cells (cirDC)) and about 60-70% granulocytes (including neutrophils, eosinophils and basophils). The characteristics of each of these cell types that facilitate their identification and isolation, including relative size, density, granularity and presence of cell surface markers, are well known in the art (see, for example, Kuby, "Immunology" 3rd ed., Freeman & Co., New York (1997)).

Cell populations described herein, containing desired percentages of different hematopoietic cell types, can be obtained by methods known in the art, including density gradient separation through media such as Ficoll or Percoll, apheresis, and positive and negative selection methods (e.g. immunomagnetic selection or flow cytometry), alone or in any combination.

Apheresis is a preferred method to remove large numbers of blood cells of a particular type (e.g. peripheral blood mononuclear cells or platelets) from an individual, while returning red blood cells. Cell separators suitable for apheresis and their uses are well known in the art, and include, for example, the FENWAL CS 3000™ cell separator (Baxter International Inc, Deerfield, Ill.), the HAEMONETICS MCS™ system (Haemonetics Corp., Braintree, Mass.), and the COBE Spectra Apheresis System™ (Gambro BCT). A preferred method of further selection of desired cell subsets is immunomagnetic selection using an automated cell selection system, such as an ISOLEX 300i™ cell selection device (Nexell Therapeutics Inc., Irvine CA). Immunomagnetic selection methods are described further below.

As used herein, the term "leukocyte population" refers to a hematopoietic cell population enriched for nucleated cells by substantially removing red blood cells from the population.

As used herein, the term "mononuclear cell population" refers to a leukocyte population from which granulocytes have been substantially removed. In a mononuclear cell population, generally at least about 30%, 40%, 50% or more, more preferably at least about 60%, 70%, 80%, 90%, 95%, or more of the leukocytes are mononuclear cells.

As used herein, the term "lymphocyte population" refers to a mononuclear cell population from which monocytes, stem and progenitor cells, and circulating dendritic cells (cirDC) have been substantially removed. In a lymphocyte population,

generally at least about 60%, more preferably at least about 70%, 80%, 90%, 95%, or more of the leukocytes are T cells, B cells and NK cells.

As used herein, the term "T lymphocyte population" refers to a lymphocyte population from which B cells and NK cells have been substantially removed. In a T lymphocyte population, generally at least about 60%, more preferably at least about 70%, 80%, 90%, 95%, or more of the leukocytes are T cells.

As used herein, the term "T lymphocyte subset" refers to a T lymphocyte population that is further enriched for a desired subset of T cells, such as CD4+ or CD8+ cells; Th1 or Th2 cells; α/β or γ/δ T cells; and the like.

Throughout this disclosure, when referring to cell surface markers (e.g. CD4 antigen and the like), the term "+" or "positive" is intended to indicate that as assessed by standard phenotyping procedures used in the immunological arts, such as FACS analysis, immunofluorescence or immunohistochemistry, the cells express the recited marker at levels similar to positive control cells. The term "-" or "negative" indicates that under the same conditions, the cells express the recited marker at levels similar to negative control cells. Exemplary methods to determine whether cells are "+" or "-" for various cell surface markers are shown in Example I, below. Antibodies to blood cell surface markers recited herein, which are suitable for phenotyping and for positive and negative selection methods, are commercially available.

In one embodiment, a peripheral blood leukocyte population is obtained from a donor administered at least one mobilizing agent. As used herein, the term "mobilizing agent" refers to an agent that increases the number of leukocytes, or any subset of leukocytes, in the blood, such as by inducing the proliferation, differentiation and/or mobilization from the bone marrow of hematopoietic stem or progenitor cells. Mobilizing agents can increase the number of leukocytes in the blood by at least 2-fold, such as at least 5-fold, including at least 10-fold or more, as compared with normal blood.

Mobilizing agents commonly used in connection with hematopoietic cell transplantation include chemotherapeutic agents, irradiation and cytokines, or any combination of these agents. Exemplary mobilizing agents include the following cytokines, alone or in any combination: ligand for the Flt3 receptor (FLT3L), cyclophosphamide, granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), stem-cell factor (SCF), macrophage colony stimulating factor (M-CSF), interleukins (e.g. IL-1 through IL-18, and the like), leukemia inhibitory factor (LIF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor beta (TGFβ), tumor necrosis factor (TNF) interferons (e.g. IFN-α, IFNβ, IFN-γ and the like), and agonists of the receptors for any of these molecules, such as daniplestim, progenipoietin (ProGP) and myelopoietin (MPO).

Mobilizing agents (and cirDC mobilizing agents described below) can be obtained from commercial sources and are of sufficient purity for human administration. Alternatively, cytokine mobilizing agents can be prepared recombinantly by methods known in the art, given that

their nucleic acid sequences are available in public databases and, further, plasmids containing the full-length sequences are commercially available. Mobilizing agents that act as agonists of cytokine receptors can be obtained commercially, designed rationally based on the known receptor structure, or obtained by screening compound libraries.

Appropriate dosages, schedules and routes for administration of mobilizing agents (and cirDC mobilizing agents described below) to individuals can be determined by a clinician, and will depend on factors such as the bioactivity of the particular agent, and the health and body weight of the individual.

The invention methods take advantage of the superior ability of recipient-derived stimulatory cell populations enriched for antigen-presenting cells, as compared with bulk peripheral blood mononuclear cells from the same individual, to activate alloreactive or antigen-reactive T cells in a donor cell population, allowing their effective depletion or isolation from a donor T cell population.

The invention methods also take advantage of the higher selectivity of recipient-derived stimulatory cell populations enriched for antigen-presenting cells, as compared with bulk peripheral blood mononuclear cells. In enriched stimulatory cell populations there are fewer hematopoietic cell surface antigens present against which donor T cells might be activated in a coculture. Accordingly, cells with the ability to react with donor hematopoietic cells (e.g. for mini-transplant applications, and to induce graft-versus-leukemia reactions) will be retained in the graft, while alloreactive T cells are effectively removed.

Preferred stimulatory cell populations for use in the methods of the invention are dendritic cell populations, and macrophage populations.

In one embodiment, the recipient-derived stimulatory cell population is a dendritic cell population. Dendritic cells (DCs) are white blood cells (leukocytes) that are specialized to present both self and foreign molecules (antigens) to the immune system. Although no single surface marker is uniquely associated with dendritic cells, DCs can be distinguished from other hematopoietic cells by their lack of expression of surface marker profiles associated with B cells, T cells, monocytes and NK cells, in combination with high expression of major histocompatibility antigens. Dendritic cells can also be distinguished from other hematopoietic cell types by their greater ability to stimulate a mixed lymphocyte reaction *in vitro*. Other distinguishing properties of dendritic cells are known in the art, and are described, for example, in Banchereau et al., Annu. Rev. Immunol. 18:767-811 (2000).

As used herein, a "dendritic cell population" refers to any hematopoietic cell population containing a higher percentage of dendritic cells than is found in the mononuclear cell portion of normal blood. Dendritic cells generally make up about 1% of mononuclear cells present in the peripheral blood of a normal, untreated individual. A "dendritic cell population" thus refers to a hematopoietic cell population in which at least 2.5%, such as at least 3%, 5%, 10%, 15%, 20% or more of the mononuclear cells are dendritic cells. Preferably, a dendritic cell population is a hematopoietic cell population containing higher percentages of dendritic

cells, such as a population in which at least 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the mononuclear cells are dendritic cells.

The methods described herein can be practiced with recipient-derived dendritic cell populations prepared by various methods. For example, a dendritic cell population can be an "uncultured dendritic cell population," which comprises DCs derived from the blood ("cirDC") without further maturation in culture. Alternatively, a dendritic cell population can be a "cultured dendritic cell population," obtained by culturing cirDC *in vitro* in the presence of maturation factors, or by culturing a monocyte cell population, an adherent mononuclear cell population, or a CD34+-cell population first in the presence of differentiation factors, and then optionally in the presence of maturation factors. Each of these dendritic cell populations has a different repertoire of expressed surface antigens, as described below, but is able to effectively activate reactive donor T cells in the methods of the invention.

Each of these cell populations will also have a different repertoire of non-dendritic cells. In applications in which it is desired to retain T cells in the donor graft with the ability to react against recipient hematopoietic cells and leukemic cells, the depletion method is preferably practiced with a dendritic cell population containing a low percentage of stem cells and other immature hematopoietic cells is preferred, such as a cultured dendritic cell population.

In one embodiment, the recipient-derived dendritic cell population comprises circulating dendritic cells. As used herein, the term "circulating dendritic

cell" or "cirDC" refers to a leukocyte present in the blood that is "lineage negative" (ie. negative for surface antigens considered in the art to be characteristic markers for T cells, B cells, monocytes, NK cells, and granulocytes, such as CD3-, CD19-, CD14-, CD56- and CD15-) and HLA-DR+.

There are two phenotypically and functionally distinct dendritic cell populations in the blood, characterized by the differential expression of the β2 integrin CD11c (see, for example, Robinson et al., Eur. J. Immunol. 29:2769-2778 (1999); Kohrgruber et al., J. Immunol. 163:3250-3259 (1999); Pulendran et al., Blood 94:213a (1999)). CD11c+ cirDC, which are considered to be more potent stimulators of allogeneic T cell proliferation than CD11c- cirDC, are preferred cirDC for use in the methods described herein.

Preferred methods of preparing a dendritic cell population comprising cirDC avoids density gradient centrifugation and positive selection steps, which could alter the functional properties of the cirDC or cause adverse effects upon administration to humans. Preferred preparation methods are also amenable to being performed in a closed fluid path system, such that the operator is not exposed to infectious agents present in the cell composition, and the cells are not exposed to environmental contaminants.

In one embodiment, the method for preparing a dendritic cell population comprising cirDC is practiced by administering to the recipient one or more agents that increase the percentage of cirDC represented among blood leukocytes, and obtaining a blood leukocyte population from the individual. Optionally, the blood leukocyte population can be further enriched for mononuclear cells,

depleted of certain non-cirDC cells, or cultured in the presence of maturation factors, as described below. Agents that increase the percentage of cirDC represented among blood leukocytes are termed herein "cirDC mobilizing agents." CirDC mobilizing agents can increase the number of cirDC among blood leukocytes by at least 2-fold, such as at least 5-fold, 10-fold, 20-fold, 30-fold or more as compared with normal blood leukocytes. Therefore, a blood leukocyte population obtained from an individual administered a cirDC mobilizing agent is a dendritic cell population comprising cirDC suitable for use in the methods of the invention.

CirDC mobilizing agents include, for example, FLT3L, G-CSF, GM-CSF, and agonists of the receptors for these cytokines, such as progenipoietin (ProGP) which is a dual receptor agonist of both the G-CSF and the flt3 receptors (Fleming et al., Blood 94:49a (1999), and thus contains an "active moiety" of both G-CSF and FLT3L. A particularly preferred mobilizing agent is FLT3L, which is the subject of U.S. Patent Nos. 5,554,512 and 5,843,423. In preclinical studies, administration of FLT3L was shown to be safe and well tolerated at doses up to 100 µg/kg/day for 14 days, and to increase cirDC levels by up to 30-fold (Lebsack et al., Blood 90:170a (1997)).

Administration of 10µg/kg/day of FLT3L for 10 consecutive days has been shown to increase cirDC in the blood that are phenotypically CD11c+IL3R- by 48-fold, and cirDC that are phenotypically CD11c-IL3R+ by 13-fold (see Pulendran et al., supra (1999)). Another preferred cirDC mobilizing agent is G-CSF. Administration of 10µg/kg/day of G-CSF for 5 consecutive days has been shown to increase cirDC that are phenotypically CD11c-IL3R+ by 7-fold (see Pulendran et al., supra (1999)).

Those skilled in the art can determine, based on factors such as the health and age of the recipient and the condition being treated, whether it is appropriate to administer to the recipient a mobilizing or cirDC mobilizing agent.

In another embodiment, the method for preparing a dendritic cell population comprising cirDC involves depleting a blood leukocyte population (e.g. a peripheral blood mononuclear cell population) of B cells and T cells, and optionally further depleting the blood leukocyte population of monocytes, granulocytes and/or NK cells.

The blood leukocyte population to be depleted of B cells and T cells can be obtained from an untreated recipient, from a recipient administered one or more cirDC mobilizing agents (described above), or from an individual administered one or more mobilizing agents, as described above with respect to donor cell populations, some of which are also cirDC mobilizing agents.

A preferred method for depleting a blood leukocyte population of B cells and T cells involves binding the desired cell with a "cell selective binding agent" so as to form a complex, and removing the bound complex from the population. However, other methods of depleting non-cirDC are known in the art or can be readily determined. Such methods, include, for example, erythrocyte rosetting (preferably using human erythrocytes), which can be used to deplete T cells; cell size or density separations (eg. counterflow elutriation), which can be used to deplete T cells, B

cells or monocytes; complement-mediated cell lysis (eg. using CAMPATH antibody), which can be used to deplete T cells or B cells; adherence to plastic, which can be used to deplete monocytes; and combinations of these methods.

As used herein, the term "cell selective binding agent" is a molecule that binds with high affinity to a molecule present on the surface of a recited hematopoietic cell, that is not also substantially present on the surface of a cirDC. Cell selective binding agents bind to molecules present at levels on the indicated cell type that are at least 10-fold, such as at least 100-fold, including at least 1000-fold higher than on cirDC.

Molecules present on the surface of B cells include, for example, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CDw75, CD76, the Ig light chains κ and λ, and the Ig heavy chains γ, α, μ, δ, and ε. Thus, a B cell selective binding agent can be a binding agent that binds any of these molecules, such as an antibody specific for any of these molecules. Preferred B cell selective binding agents bind to CD19, CD20, CD21, CD22 or CD37. Particularly preferred B cell selective binding agents bind to CD19 or CD20.

Molecules present on the surface of T cells include, for example, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD27, CD28, CD32, CD43, and the T cell receptor α, β, γ or δ chains. Thus, a T cell selective binding agent can be a binding agent that binds any of these molecules, such as an antibody specific for any of these molecules. Preferred T cell selective binding agents bind to CD2, CD3, CD4, CD5, CD7, CD8, or the TCR α or β chains. Particularly preferred T cell selective binding agents bind to CD2 or CD3.

Molecules present on the surface of monocytes include, for example, CDw12, CD13, CD14, CD15, CDw17, CD31, CD32, CD33, CD64 and CD98. Thus, a monocyte selective binding agent can be a binding agent that binds any of these molecules, such as an antibody specific for any of these molecules. A preferred monocyte selective binding agent binds to CD14.

Molecules present on the surface of granulocytes include, for example, CD66b, CD15 and CD24. Thus, a granulocyte selective binding agent can be a binding agent that binds any of these molecules, such as an antibody specific for CD66b, CD15, or CD24. Depleting a blood leukocyte population of granulocytes using a granulocyte selective binding agent is particularly advantageous when the starting blood leukocyte population contains a significant number of mature or immature granulocytes. For example, when blood is obtained from an individual administered a mobilizing agent such as G-CSF, GM-CSF, or progenipoietin (ProGP), a blood leukocyte population can contain a large number of mature and immature granulocytes. Immature granulocytes can be difficult to separate from mononuclear cells using cell separators, but can advantageously be depleted using a granulocyte selective binding agent.

Molecules present on the surface of NK cells include, for example, CD16 and CD56. Thus, an NK cell selective binding agent can be a binding agent that binds any of these molecules, such as an antibody specific for CD16 or CD56.

A binding agent useful in the methods of the invention will form a high affinity binding complex with the target cell. As used herein, the term "complex" refers to an interaction between the binding agent and

the target cell that has a dissociation constant (Kd) of less than about 10^{-5} M, such as less than about 10^{-7} M, including less than about 10^{-9} M. A preferred binding agent is an antibody, such as a monoclonal, recombinant or single chain antibody, or an antigen binding fragment therefrom, which forms a high affinity complexes with target molecules. Antibodies suitable for use in the methods of the invention are commercially available, or can be produced with high affinity for a desired surface molecule by methods known in the art. Such antibodies can be derived from a single species, including human, rodent, sheep and goat, or can be chimeric.

Preferred cell selective binding agents bind to all or to the majority of the indicated cell type. However, combinations of cell selective binding agents can be used to more completely deplete (or isolate) a particular cell type. As an example, CD4 is expressed on about 65% of T cells, with the remainder expressing CD8. Thus, a combination of binding agents that bind CD4 and CD8 can be used to deplete T cells.

Cell selective binding agents other than antibodies can also be used in the methods of the invention. Such binding agents include lectins, such as soybean agglutinin, which binds to T cells and B cells. Commercially available libraries of small molecule or macromolecular compounds can also be screened using whole B cells, T cells or monocytes, or membranes or isolated surface molecules therefrom, to identify other appropriate binding agents. Methods of screening and selecting for binding compounds, including automated screening and selection methods, are well known in the art. The particular method employed will depend on the nature of the compounds being screened. Thus, a cell selective binding agent can be essentially any chemical

or biological compound with the appropriate selectivity and affinity for the desired cell type, such as a nucleic acid, peptide, peptidomimetic, small organic molecule, or the like.

Methods of preparing a dendritic cell population comprising cirDC, by depleting a blood leukocyte population of various non-cirDC cells, are described in detail in U.S. patent application serial no. 09/578,532, which is incorporated herein by reference.

Alternative methods of preparing a dendritic cell population comprising cirDC are known in the art. For example, Robinson et al., Eur. J. Immunol. 29:2769-2778 (1999)), describes a method involving subjecting buffy coats to serial density gradient centrifugation, followed by immunomagnetic depletion of B cells, T cells, monocyte and NK cell populations using CD3, CD14, CD20 and CD16 antibodies. Kohrgruber et al., J. Immunol. 163:3250-3259 (1999), describes a method involving FICOLL separation of an apheresis product followed by counterflow elutriation to remove debris and small lymphocytes. The pooled elutriation fractions were immunomagnetically depleted of T, B, NK, hematopoietic stem cells and monocytes using a cocktail of anti-CD3, CD11b, CD16, CD19, CD34 and CD56 antibodies. Miltenyi Biotec (Gladbach, Germany) sells a blood dendritic cell isolation kit for magnetically depleting T cells, monocytes and NK cells by retention on a column, followed by positive selection of CD4+ blood dendritic cells using CD4 microbeads.

Methods of preparing a dendritic cell population comprising cirDC using positive selection with cirDC-selective binding agents are also known in the art. For example, monoclonal antibodies have been developed by

Miltenyi Biotec (Gladbach, Germany) against antigens present on the surface of cirDC, which are designated BDCA-2, BDCA-3 and BDCA-4 (Schmitz et al., Abstracts from the 6th International Symposium on Dendritic Cells, available online at http://www.wehi.edu.au/dc2000/, p. 23 (2000)). Maturation of cirDC in culture results in the upregulation of BDCA-3 and BDCA-4, and the downregulation of BDCA-2. The BDCA-4 surface marker is also present on the surface of monocyte-derived dendritic cells and CD34+-derived dendritic cells. Using binding agents specific for such markers, positive selection methods, including, for example, immunomagnetic selection and FACS sorting, can be used to isolate cirDC.

Optionally, a dendritic cell population comprising cirDC can be cultured in a medium containing at least one factor that promotes the maturation of dendritic cells, as described further below.

In an alternative embodiment, the recipient-derived dendritic cell population comprises cultured dendritic cells produced by a method comprising culturing a recipient-derived mononuclear cell population in medium containing at least one factor that promotes the differentiation and/or maturation of dendritic cells. Methods of preparing cultured dendritic cells from mononuclear cells present in peripheral blood, bone marrow or umbilical cord blood are known in the art and described, for example, in WO 98/06823 and WO 98/06826, which are incorporated herein by reference. Mononuclear cells can be obtained from an untreated recipient, or from a recipient administered one or more mobilization agents, as described above.

Preferably, the culture medium for preparing cultured dendritic cells will be serum-free, and optionally also animal-protein free. The absence of animal proteins (e.g. bovine serum albumin) prevents contact with foreign proteins that could detrimentally affect the function of the cells, and also reduces the risk of immune responses developing in the recipient. The culture medium optionally contains clinical grade human serum albumin in a concentration of about 0.5 - 5.0%, usually about 1.0% (w/v) (e.g. BUMINATE™, Baxter Hyland, Glendale, CA).

A suitable base culture medium for preparing cultured dendritic cells can be of any formulation favorable for the growth and/or maintenance of hematopoietic cells. Exemplary media include X-VIVO 10™ or X-VIVO 15™ medium (BioWhittaker, Walkersville, MD), Hematopoietic Stem Cell-SFM medium (GibcoBRL, Grand Island, NY), Iscove modified Dulbecco medium (IMDM), AIM V (Gibco, BRL) and RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO). Other appropriate cell culture media are well known in the art.

In one embodiment, the starting cell population for preparing cultured dendritic cells is a monocyte cell population. As used herein, the term "monocyte" refers to a leukocyte that is phenotypically CD14+, and does not express T cell and B cell markers (e.g. is CD3- and CD19-). As used herein, a "monocyte cell population" refers to a mononuclear cell population from which B and T lymphocytes have been substantially removed. In a monocyte population, generally at least about 40%, 50% or 60%, more preferably at least about 70%, 80%, 90%, 95%, or more of the leukocytes are monocytes. A monocyte cell population can be prepared by methods known in the art, such as by depleting a PBMC population of T cells and B

cells (see Example, below), or by elutriation of a PBMC population (see, for example, Rouard et al., J. Immunol. Meth. 241:69-81 (2000)).

In another embodiment, the starting cell population for preparing cultured dendritic cells is an "adherent mononuclear cell population." To prepare an adherent mononuclear cell population, mononuclear cells are first incubated in a polystyrene culture container for a time sufficient to allow a subset of cells to adhere to the inner surface of the container monocytes. Preferably, the incubation time is at least 1 hour, more preferably at least 2 to 4 hours, up to about 18 hours. After incubation, the non-adherent cells are removed, and those cells which adhere to the polystyrene inner surface are further cultured as described below to prepare a dendritic cell population.

In culture methods in which the starting cell population is a monocyte cell population or an adherent cell population, preferred dendritic cell differentiation factors are GM-CSF, in combination with either IL4, IL13 or IL7. Preferred culture time periods are from about 5 to about 10 days, under standard cell culture conditions. Dendritic cells prepared from monocytes and adherent mononuclear cells can be characterized by their characteristic scatter profile (see Figure 1A), by being negative for expression of cell surface markers characteristic of T cells, B cells and monocytes (e.g. CD3-, CD19-, and CD14-), and also by being positive for expression of HLA-DR.

In a further embodiment, the starting cell population for preparing cultured dendritic cells is a "CD34+ cell population." A CD34+ cell population is substantially free of non-CD34+ cell types, and is

preferably prepared by positive selection of CD34+ cells, using methods well known in the art, such as immunomagnetic selection using an ISOLEX 300i™.

In culture methods in which the starting cell population is a CD34+ cell population, preferred dendritic cell differentiation factors are GM-CSF in combination with TNFα, and optionally further in combination with SCF or FLT3L. Preferred culture time periods are from about 5 to about 10 days, under standard cell culture conditions. Dendritic cells prepared from a CD34+ cell population can be characterized as cells which are CD14+ or CD1a+, and HLA-DR+.

Optionally, the uncultured cirDC or cultured DC populations, prepared as described above, are cultured in a medium containing at least one maturation factor for a period of from about 1 to 3 days, in order to produce mature DCs. Mature DCs differ from immature DCs in their reduced ability to take up antigen by pinocytosis, phagocytosis and endocytosis, their enhanced ability to present antigen, and their enhanced ability to stimulate T cell activation. Additionally, the expression of surface markers, including CD83, CD86 and CD80, is higher on mature DCs than on immature DCs. Other functional and phenotypic characteristics of mature and immature DCs are well known in the art, and are reviewed, for example, in Banchereau et al., supra (2000).

Those skilled in the art can determine whether it is more appropriate to use mature DCs or immature DCs in the methods of the invention. For example, either immature or mature DCs will effectively stimulate alloreactive donor T cells. For such applications it may be preferred to use immature DCs, as their preparation is more rapid. Likewise, either immature or mature DCs are

suitable for use in applications in which the DCs are pulsed with antigens that do not need to be processed, such as peptide antigens. For other applications, such as when the DCs are to be pulsed with antigens that require processing (e.g. proteins, cells and extracts), immature DCs may be preferred. Antigen-pulsed immature DCs can, if desired, be further matured in culture.

Exemplary maturation factors suitable for maturing DCs include calcium ionophore, TNFα, prostaglandin E (PGE), monocyte conditioned medium, IFNα, CD40L and lipopolysaccharide (LPS), which are preferably used in combination with a differentiation factor such as GM-CSF.

In the methods of the invention, the recipient-derived dendritic cell population co-cultured with the hematopoietic donor cells can be alive and proliferating. Preferably, however, the recipient-derived dendritic cell population is pre-treated with at least one agent that prevents the cells from proliferating while preserving their stimulatory activity. Exemplary agents that prevent the cells from proliferating include irradiation and mitomycin C.

The recipient-derived dendritic cell population and the donor cell population are co-cultured at a ratio of from about 1:5 to about 1:500, such as about 1:10, 1:50, 1:75, 1:100, 1:150, 1:200 or 1:400, for a period of time sufficient for activation of reactive T cells within the donor cell population. A sufficient time period can be determined by observing the kinetics of expression of activation markers, as described below. Generally, a sufficient time period to induce expression of activation markers is at least 12 h, more preferably at least 24 h or 48 h, including at least 3, 5, 7, or 10 days.

In another embodiment, the recipient-derived stimulatory cell population is a macrophage population, such as a population of tissue macrophage or cultured macrophages. Tissue macrophages are named to reflect their tissue origin, such as alveolar macrophages in the lung, histiocytes in connective tissues, and the like, and can be obtained by methods known in the art. Because of their ease of preparation, a recipient-derived macrophage population is preferably a cultured macrophage population.

As used herein, the term "cultured macrophage" refers to a CD14+CD86+ cell that is derived from a monocyte in culture. A macrophage can be distinguished from a monocyte by its larger size, as well as by the enhanced expression of CD86 surface antigen. A macrophage can be distinguished from a monocyte-derived dendritic cell by the expression of CD14 surface antigen. The term "cultured macrophage population" refers to a population in which at least 10%, preferably 20%, 40%, 60%, 80%, 90% or more of the starting monocytes have been differentiated in culture into macrophages. As described above with respect to dendritic cells, in applications in which it is desired to retain T cells in the donor graft with the ability to react against recipient hematopoietic cells and leukemic cells, a highly enriched cultured macrophage population is most preferred, such as a population containing at least about 70% macrophages.

Methods of differentiating monocytes along the macrophage lineage are well known in the art. For example, monocytes can be differentiated into macrophages by culture of monocytes in GM-CSF alone, or M-CSF alone, or autologous serum, for several days. Macrophages can be activated so as to increase their potency by culture in a combination of GM-CSF and either IFNγ or Vitamin D3,

or both. Exemplary methods for preparing a macrophage population are described in Example II, below, and in Boyer et al., Exp. Hematology 27:751-761 (1999), and Andreesen et al., J. Leukocyte Biol. 64:419-424 (1998). Preferred media formulations and co-culture conditions have been described above with respect to dendritic cell populations.

As shown in Figure 5, recipient-derived macrophages are able to effectively stimulate alloreactive donor T cell proliferation in a mixed lymphocyte reaction, particularly at a ratio of recipient-derived macrophages to donor T cells of about 1:10 to about 1:5. Because T cell proliferation in a MLR is highly correlated with activation marker expression, these results indicate that a recipient-derived macrophage population will be an effective recipient-derived stimulatory cell population for use in the methods of the invention.

The methods of the invention are practiced by coculturing the recipient-derived stimulatory cell population with a hematopoietic donor cell population so as to activate donor T cells, and killing or removing the activated T cells, thereby depleting (or isolating) reactive T cells from the donor cell population. As used herein, the term "reactive T cell" refers to a T cell present in a donor hematopoietic cell population that has the potential to recognize, become activated and proliferate in response to an alloantigen (producing an "alloreactive T cell"), or other antigen (an "antigen-reactive T cell") present on the surface of, or presented by, the recipient-derived stimulatory cell population.

In allograft procedures, donor T cells are reactive with recipient alloantigens. As used herein, the term "alloantigen" refers to class I and class II major histocompatibility (MHC) or HLA antigens, as well as minor histocompatibility antigens, that differ between individuals, and which are naturally present on the surface of cells in the recipient-derived stimulatory cell population. The methods of the invention can be practiced with individuals who are closely HLA-matched, sharing all or nearly all of their class I and class II HLA antigens; haploidentical, such as siblings sharing half of their HLA antigens; or unrelated, and thus poorly HLA matched. The degree of HLA identity between individuals can readily be demonstrated by methods known in the art, including the polymerase chain reaction, mixed lymphocyte reactions (MLR), and serological measurements. In allograft procedures, the methods of the invention can thus be used to activate and deplete donor T cells with the potential to react with alloantigens present on the surface of recipient-derived stimulatory cells, so as to reduce the risk of the recipient developing graft-versus-host disease.

Donor T cells also react with antigens presented by recipient-derived stimulatory cells. As used herein, the term "antigen" refers to any antigen (other than an alloantigen) against which it may be beneficial to establish an activated reactive T cell population, or against which a reactive T cell population in a hematopoietic stem cell graft may be detrimental. In the methods of the invention, the stimulatory cell population can first be co-incubated with antigen, or "pulsed," and the pulsed cells cocultured to activate antigen-reactive T cells in the hematopoietic donor cell population, which can then be depleted or isolated as described below.

Methods of pulsing antigen-presenting cells are well known in the art. In an exemplary method, the stimulatory cell population, at a concentration of several million/ml, is co-incubated with antigen, at a concentration of about 10-200 μg/ml, for a period of from several hours to several days.

In applications in which a graft is to be used in the treatment of an autoimmune disease, it may be beneficial to deplete autoantigen-reactive T cells from the graft. As used herein, the term "autoantigen" refers to a host antigen (or microbial superantigen) considered by those skilled in the art to be associated with an autoimmune disease, such that the presence of activated T cells specific for the autoantigen is correlated with development or progression of the disease.

Human autoimmune disorders include, but are not limited to, neurological disorders such as multiple sclerosis (MS) and amyotrophic lateral sclerosis; rheumatological disorders such as rheumatoid arthritis (RA), systemic sclerosis, juvenile rheumatoid arthritis and systemic lupus erythematosus; and other disorders including pemphigus vulgaris, psoriasis, diabetes, myasthenia gravis, thyroiditis, scleroderma, thrombocytopenic purpura, cryoglobulinemia, autoimmune haemolytic anemia, and insulin-dependent diabetes mellitus (IDDM). Animal models of human autoimmune disorders include, but are not limited to, experimental autoimmune encephalomyelitis, which is a model of MS; collagen-induced arthritis and adjuvant-induced arthritis, which are models of RA; and non-obese diabetes (NOD mice), which is a model of IDDM.

For several of these disorders, autoantigens have been identified. For example, myelin-derived antigens, including myelin basic protein and proteolipid protein, are associated with MS; desmoglein3 is associated with pemphigus vulgaris; glutamic acid decarboxylase is associated with diabetes; and acetylcholine receptor is associated with myasthenia gravis. For other autoimmune disorders, in which individual autoantigens have not yet been characterized, an autoantigen suitable for practice of the methods of the invention can be cells or a cell extract from the affected tissue (e.g. synovial cells for rheumatoid arthritis, a skin lesion for psoriasis, etc.).

In applications in which the graft is to be used to treat malignancies and infectious conditions, it may be beneficial to isolate and administer activated T cells reactive toward malignant and infected cells. For such applications, appropriate antigens can include whole target cells; lysates of the cells; pathogens or components thereof; as well as isolated molecules, or any combination thereof.

Antigens suitable for use in preparing activated T cells for use in therapy of various different types of cancers are known in the art and include, for example, carcinoembryonic antigen (CEA) (e.g. for breast or colon cancer); Her2/neu (e.g. for breast or ovarian cancer); prostate specific antigen (PSA), prostate specific membrane antigen (PMSA), and prostatic acid phosphatase (e.g. for prostate cancer); MUC (e.g. for colorectal, pancreatic, ovarian, lung and breast cancer); thyroglobulin (e.g. for thyroid cancer); MAGE, GP100, GAGE tyrosinase or MART1 (e.g. for melanoma); T cell receptor (e.g. for T cell non-Hodgkin's lymphoma); immunoglobulin idiotype (e.g. for B-cell non-Hodgkin's

lymphoma and multiple myeloma); mutant p53 (e.g. for colorectal, lung, bladder and head and neck cancers); and p210/bcr-abl fusion product (e.g. CML and ALL).

Antigens suitable for use in preparing activated T cells for use in therapy of various infectious conditions are also known in the art and include, for example, human immunodeficiency virus (e.g. HIV-1 and HIV-2), hepatitis B virus, hepatitis C virus, papilloma virus, cytomegalovirus, Epstein-Barr virus, and chlamydia, as well as antigenic preparations therefrom, as well as cells infected with such viruses and lysates therefrom.

Co-culturing of a recipient-derived stimulatory cell population (such as an allogeneic or antigen-pulsed DC or macrophage population) with a hematopoietic donor cell population results in the activation of T cells within the donor cell population. The methods of the invention for depleting reactive T cells take advantage of the expression of "activation markers" which are characteristic of activated T cells.

For example, activated T cells generally express one or more "surface activation markers," which are molecules that are expressed, or expressed in higher abundance, on the surface of T cells following activation. Exemplary activation markers include CD25 (IL2 receptor), CD69, CD38, CD71 (transferrin receptor) and CD134 (OX40). The structural and functional properties of T cell activation markers, as well as reagents suitable for detecting such markers, are well known in the art (see, for example, Barclay et al., "The Leucocyte Antigen FactsBook," Academic Press, San Diego, CA (1993)).

Activated T cells also produce a number of "secreted activation markers," which are molecules that are secreted, or secreted in higher amount, following activation. Exemplary secreted activation markers include, for example, cytokines such as interleukin-2 (IL2), IL4, IL5, and γ-interferon (IFNγ). The structural and functional properties of activation cytokines, as well as reagents suitable for detecting such cytokines, are well known in the art (see, for example, Thomson, ed., "The Cytokine Handbook," 2nd ed., Academic Press Ltd., San Diego, CA (1994)).

In order to deplete reactive T cells, following activation, the co-cultured cells are contacted with one or more binding agents that bind to T cell activation markers so as to form a complex. In order to apply such methods with respect to secreted activation markers, affinity matrix methods have been developed in which a bivalent binding agent with specificity for both the secreted molecule and a cell surface molecule are allowed to contact the T cells, after which the secreted molecule, relocated to the affinity matrix, is contacted with a binding agent (see, for example, WO 99/58977). For example, Brosterhaus et al., Eur. J. Immunol., 29:4053-4059 (1999), describes a method of detecting activated T cells by first contacting the T cells with an antibody-antibody conjugate directed against both CD45 and IFNγ, allowing the cells to secrete IFNγ for 45 min, after which the IFNγ-secreting cells are contacted with a PE-conjugated IFNγ-specific antibody. Bivalent agents for binding to both T cells and secreted activation markers can be prepared by methods known in the art, such as methods described in WO 99/58977, and are commercially available, for example, from Miltenyi Biotec (www.miltenyibiotec.com).

As used herein, the term "depleting" refers to any method of treating the population of donor cells such that it contains fewer reactive T cells after treatment than before treatment. Preferably, the removal method is efficient, such that at least 50% of the reactive T cells are removed from the donor population, and more preferably at least 60%, 70%, 80%, 90%, 95%, 98% or more. Additionally, preferably the removal method is selective, such that at least 50% of the T cells removed from the donor population are reactive T cells, and more preferably at least 60%, 70%, 80%, 90%, 95%, 98% or more.

The term "depleting" is intended to include, for example, physical removal of the reactive T cells from the population of donor cells, as well as killing (or inactivation) of the reactive T cells. Physical removal is preferred when it is desirable to also isolate the activated, reactive T cells. As used herein, the term "isolating" is intended to refer to the process of collecting, separate from the starting hematopoietic donor cell population, the depleted activated T cells.

Binding agents for physical removal procedures can optionally be conjugated directly or indirectly (e.g. via a secondary antibody or binding agent) to a labeled moiety suitable for the particular separation procedure. For example, for immunomagnetic separation, the labeled moiety can be magnetic particle (e.g. a bead). For FACS separation, the labeled moiety can be a fluorophore. However, for other separation procedures, such as affinity binding and density gradient separation procedures, the binding agent need not be labeled. Appropriate binding agents, labeled moieties, and methods for physical removal of bound cells are well known in the art. In a preferred embodiment, the physical removal method is practiced by contacting the coculture with a

primary antibody directed against a cell surface activation marker, followed by a magnetically labeled secondary antibody, and immunomagnetically removing the bound complex from the co-culture.

Binding agents suitable for killing activated reactive T cells binding agents conjugated directly or indirectly (e.g. via a secondary antibody or binding agent) to a toxin. Appropriate toxins include radioisotopes, as well as chemotoxic agents, such as ricin, and chemostatic agents.

It is contemplated that the methods of the invention can be used in combination. For example, for preparing an allograft enriched for antigen-reactive T cells, which has a reduced ability to cause GVHD, it may be advantageous to first activate and deplete alloreactive T cells from the donor cell population, and then activate and isolate antigen-reactive cells, or perform the steps in the reverse order. It will also be appreciated that the methods of the invention can simultaneously activate and deplete both alloreactive and autoantigen-reactive T cells from a donor allograft.

Preferably, all steps from obtaining cells from the donor and recipient, to infusing the therapeutic composition into the recipient, are carried out in a closed fluid path system. For example, peripheral blood from an individual can be collected and separated using an automated blood cell separator such as the CS3000™ cell separator (Fenwal Division, Baxter Healthcare, Deerfield, Ill.), which can be asceptically connected to an ISOLEX 300i™. If desired, samples of cells at any stage can be aseptically drawn off from the container system through sterile-connect ports for analysis. In methods involving antigen pulsing of stimulatory cells,

antigens can be aseptically added to the closed fluid path system through sterile-connect ports. Culturing and co-culturing methods can be practiced in closed culture containers, such as the PL2417 (X-FOLD™) culture bag (Nexell Therapeutics Inc.) described in PCT US95/13943, which can be aseptically connected to the closed fluid path system. Finally, concentration of hematopoietic donor cell population into an infusible medium such as PLASMA-LYTE A™ (Baxter IV Systems, Round Lake, IL) can be carried out in the closed fluid path system, and the concentrated cells can be infused directly via the patient's intravenous line without exposing the cells to the environment.

The invention also provides selectively depleted, or selectively isolated, hematopoietic donor cell populations, prepared by the methods described above, as well as methods of using such compositions therapeutically in hematopoietic transplantation methods.

In one embodiment, a hematopoietic donor graft, depleted of alloreactive T cells by the methods of the invention, is administered to an allogeneic recipient in need of a graft. In such a method, the risk of developing GVHD, and the severity of GVHD if it develops, is significantly reduced compared with administering an untreated allograft.

In another embodiment, a hematopoietic donor graft, depleted of alloreactive T cells by the methods of the invention, is administered to an allogeneic recipient in order to elicit a graft-versus-leukemia cell reaction.

In yet another embodiment, a hematopoietic donor graft, depleted of alloreactive T cells by the methods of the invention, is administered to an

allogeneic recipient in order to elicit a graft-versus-hematopoietic cell reaction to ablate the recipient's hematopoietic system (ie. a "mini-transplant" procedure or "donor lymphocyte infusion" (DLI)). Mini-transplant procedures are described, for example, in Carella et al., Bone Marrow Transplantation 25:345-350 (2000). In such procedures, the recipient has generally been previously treated with a non-myeloablative, or partially myeloablative, regimen of chemotherapy or radiation.

In certain therapeutic applications, it is desirable to mount an anti-recipient cell immune reaction. Thus, in one embodiment, a donor cell population enriched for activated T cells reactive toward pathogenic host cells (e.g. reactive with antigens associated with malignant cells or infected cells) is administered to a recipient for the treatment of a pathology (e.g. cancer or an infectious disease). Advantageously, if the donor is an allogeneic individual, the graft can be depleted of allogeneic T cells, by the methods described herein, either before or after isolation of antigen-reactive T cells, to reduce the risk of developing GVHD.

For the therapy of autoimmune disease using hematopoietic stem cell transplantation, it is desirable to remove autoreactive T cells from the donor graft. Thus, in one embodiment, a donor cell population depleted of autoantigen reactive T cells is administered to a recipient for the therapy of an autoimmune disease. Advantageously, if the donor is an allogeneic individual, the graft can also be depleted of allogeneic T cells, by the methods described herein, to reduce the risk of developing GVHD. Hematopoietic stem cell transplant

procedures for the treatment of autoimmune diseases are described, for example, in Tyndall et al., supra (1999); and Fassas et al., supra (2000).

Those skilled in the art will be able to determine other suitable applications for the donor cell populations prepared by the methods of the invention.

In transplantation methods using compositions prepared by the methods of the invention, it is contemplated that administration of the graft will result in some ameliorative effect, such as improved overall immune function or a specific immune function; an improvement in the quality of life; a reduction in the severity of the symptoms of the disease; a reduction in the number of tumor cells, infected cells, leukemic cells or autoimmune cells; prolonged survival, and the like. Indicators of beneficial effect are well known in the art, and an appropriate indicator for a particular application can be determined by the skilled person.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

Comparison of the Efficacy of Recipient-Derived DCs and PBMC for Activating Reactive Donor T Cells

This example shows that recipient-derived dendritic cells are superior to peripheral blood mononuclear cells in activating reactive donor T cells, thereby allowing reactive donor T cells to be more effectively and more specifically depleted (or isolated) from the donor cell population.

Materials and Methods

Reagents

Reagents were obtained from the following vendors. IL-4 (R&D, Minneapolis,MN), GMCSF (Immunex, Seattle, WA), X-VIVO 15, PBS (Biowhittaker, Walkersville, MD), anti-CD3-PC5, anti-CD25-PC5 (Immunotech), anti-CD3-PC5, anti-CD69-PE, anti-CD14-PE, anti-CD80-PE, anti-HLA-DR-PE (Becton Dickinson, San Jose, CA). Anti-CD25-PE, anti-CD134-PE, anti-CD1a-FITC (Pharmingen, San Diego). Anti-CD86 (Ancell, Bayport, MN) RPMI.1640, HEPES, 2-mercaptoethanol (Gibco-BRL, GrandIsland, NY). AB serum (Gemini Bioproducts, Woodland, CA), Anti-CD25 mAb (Diaclone), Anti-CD2, anti-CD19 mAbs (Nexell Therapeutics Inc.) Albumin, sodium Citrate (Baxter). Sheep anti-mouse IgG-conjugated paramagnetic beads (Dynal).

Human Blood Donors

Blood or apheresis products were obtained from unrelated normal volunteer donors. Donors were arbitrarily designated and represented as either the transplant donor or the transplant recipient for this study. Blood for small-scale experiments (100-200 million PBMC) and apheresis product for large-scale (1-10 billion PBMC) experiments were used.

Generation of Dendritic Cells

DCs were generated from peripheral blood monocytes as follows. Monocytes were enriched from a portion of a normal unmobilized donor apheresis product (4 X 10^9 MNC) by depleting T and B cells on the ISOLEX 300i™. Peripheral blood mononuclear cells were suspended

in phosphate buffer saline (PBS) containing 1% human serum albumin and 0.48% sodium citrate. Cells were sensitized with 1 mg each of anti-CD2 and anti-CD19 antibodies together for 15 minutes at room temperature, in an ISOLEX 300i™ cell selection device. Unbound antibodies were removed by washing and the antibody sensitized cells were incubated with sheep anti-mouse antibody conjugated paramagnetic beads (Dynal) at a 2 beads:1 PBMC ratio for 30 minutes at room temperature. The bead/cell rosettes were washed and the unbound cells were collected, washed and resuspended in culture media.

The resulting monocyte enriched cell population (1×10^9 cells, 60% monocytes) was cultured at a cell density of 1 million cells/ml in 175 Cm^2 culture flasks (150×10^6 cells/flask)) with GM-CSF (2500 U/ml) and IL-4 (1000 U/ml) for 7 days using serum free X-VIVO 15 medium to generate DCs. Cultured cells were stained with a panel of typical DC markers (scatter parameters, expression of CD1a, CD14, CD80, CD86 and HLA-DR) and analyzed by flow cytometry for their phenotypic characterization.

Mixed Lymphocyte Reaction

For small-scale experiments shown in Figures 2-4, peripheral blood mononuclear cells (PBMC) were prepared by Ficoll gradient separation, following routine methods. For large-scale procedures, a leukapheresis product was used as the source of PBMC. PBMC from the donor were mixed with irradiated (3000 rad) recipient-derived stimulator cells in a one-way primary mixed lymphocyte reaction. Irradiated recipient PBMC or irradiated recipient-derived DCs were used as stimulator cells. The ratio of responder PBMC to irradiated recipient PBMC was 1:1 while the ratio of responder PBMC

to recipient-derived DC was 10:1. The cells were co-cultured in RPMI/5% AB serum either in 6-well plates (10 x 10^6 cells/well) or in a LIFECELL bag (160 x 10^6 donor cells/bag) for 7 days at 37°C.

Flow Cytometry

The expression of activation markers CD25, CD69, CD134 following stimulation with recipient-derived PBMC or DC was evaluated using commercially available antibodies specific to these markers. Combinations of FITC-conjugated, PE-conjugated, and PC5-conjugated (phycoerythrin-Cyanine-5) antibodies were used for three-color analysis. An aliquot of cells (1-5 million) was suspended in PBS/0.1% albumin (PAH), mixed with a predetermined concentration of specific antibodies, and incubated at room temperature for 15 minutes for staining. The cells were washed twice with PAH, fixed with 0.5% paraformaldehyde, stored at 2-8°C and analyzed within seven days. Staining was analyzed on a FACSort equipped with an argon laser using CellQuest software (Becton Dickinson). Profiles of CD25, CD69, and CD134 staining were determined on $CD3^+$ gated populations.

Cell selection procedure

For small-scale depletion (30-50 million cells), cells from MLR cultures were suspended (50 million cells/ml) in separation buffer (PBS containing 1% human albumin and 0.48% Sodium Citrate buffer) and incubated at room temperature with predetermined optimal concentration of anti-CD25 mAb (10 mg/ml) for 30 minutes while rocking. The cells were washed twice with separation buffer to remove unbound antibody and resuspended in one ml of sheep anti-mouse IgG paramagnetic bead suspension (DYNABEADS). The bead-cell

suspension was incubated at room temperature for 30 minutes with rocking. The bead-cell rosettes were retained on a magnet and the unrosetted, non-target cells ($CD25^-$) were collected.

For large-scale experiments, the ISOLEX 300i™ cell selection device (Nexell Therapeutics Inc.) was used for cell selection as follows, with similar results. All cell preparation and washing of cells in the ISOLEX™ is done automatically by an integrated cell washer, which includes a "Spinning Membrane" disposable centrifugal attachment. The ISOLEX™ uses disposable sets that are gamma-sterilized, and contains multiple filters to keep microbes from entering the flowpath. The large primary magnet and chamber is engineered to perform cell capture and release, while the octagonal secondary magnet chamber is designed to remove any remaining beads prior to final cell collection. The cells from MLR cultures in Lifecell bags were transferred to the ISOLEX™ within a closed system. Anti-CD25 mAb (0.8 mg) and sheep anti-mouse IgG DYNABEADS™ beads (1 vial, containing 4 x 10^9 beads) were added to the system and depletion were completed in approximately two hours, giving the depleted cell population in a bag.

Restimulation of depleted and non-depleted cells

Cells recovered from MLR cultures were washed with fresh culture media and divided into two aliquots. One aliquot was subjected to $CD25^+$ T cell depletion while the other was left untreated. Both aliquots of cells were separately cocultured in RPMI/5% AB serum with either irradiated recipient PBMC or DC in 6-well plates (10 x 10^6 cell/well) at 2 x 10^6 cells/ml. The ratio of responder PBMC to irradiated recipient PBMC was 1:1 while the ratio of responder PBMC to recipient-derived DC was

10:1. After 72 h in the culture, the cells were double-stained with anti-CD3-PC5 and anti-CD25-PE monoclonal antibodies and responder T cells were analyzed for CD25 expression by flow cytometry.

Results

The method of generating dendritic cells from monocytes, described above, typically resulted in the recovery of about 7 X 10^8 cells, of which about 80% were dendritic cells, as defined by scatter profile, lack of expression of CD14 and expression of HLA-DR, and further by expression of CD1a, CD86 and CD80 surface markers. FACS analysis of dendritic cell markers from a representative experiment is shown in Figure 1A-1F.

Recipient-derived DCs, or recipient-derived PBMCs, prepared as described above, were irradiated and co-cultured with donor PBMCs for 7 days, after which the expression of the surface activation markers CD25, CD69 and CD134 was assessed by FACS analysis. As shown in Figure 2, recipient-derived DCs were much more potent activators of donor T cells. For example, 23.2% of donor T cells were CD25+ following stimulation with recipient-derived DCs (Figure 2D), compared with only 9.7% after stimulation with recipient-derived PBMCs (Figure 2A). Additionally, 11.0% of donor T cells were CD69+ following stimulation with recipient-derived DCs (Figure 2E), compared with only 4.4% after stimulation with recipient-derived PBMCs (Figure 2B). Furthermore, 4.0% of donor T cells were CD134+ following stimulation with recipient-derived DCs (Figure 2F), compared with only 2.4% after stimulation with recipient-derived PBMCs (Figure 2C).

The activated donor cells were then subjected to depletion with mouse anti-CD25 antibodies and anti-mouse IgG immunomagnetic beads. As shown in Figures 3B and 3D, following depletion essentially no CD25+ T cells remained in the either donor cell population. This result demonstrates that all activated T cells can be effectively removed by immunomagnetic depletion methods.

In order to determine whether residual reactive T cells remained in the donor cell population following stimulation with DCs or PBMCs and depletion of CD25+ T cells, the depleted cell populations were restimulated with either recipient-derived DCs or PBMCs for 72 h. As shown in Figure 4C, 12% of T cells originally stimulated with recipient-derived PBMCs and then depleted of CD25+ cells were able to be activated to express CD25+ by re-stimulation with recipient-derived DCs. This result indicates that allostimulation by PBMC is not sufficient to stimulate weakly alloreactive T cells which can, however, be stimulated by DCs.

As shown in Figure 4D, essentially no T cells (0.7%) within the population originally stimulated with recipient-derived DCs and then depleted of CD25+ cells, were able to be activated to express CD25+ by re-stimulation with recipient-derived DCs. The percentages of CD25+ cells in the non-depleted population of T cells originally stimulated with recipient-derived PBMCs and then restimulated with DCs, or originally stimulated with recipient-derived DCs and then restimulated with DCs, are shown in Figures 4A (35.9%) and 4B (54.7%), respectively.

These results demonstrate that stimulation by DCs can activate reactive donor T cells more effectively than can PBMCs, such that both strongly and weakly reactive T cells can be depleted (or isolated) from the

donor cell population. In contrast, using PBMCs as the stimulators, weakly reactive T cells remain in the donor cell population following depletion of activated T cells.

The therapeutic implications of these results are first, that stimulation with DCs will allow more effective depletion of alloreactive T cells from an allograft than will stimulation with PBMCs, which should decrease the likelihood of developing graft-versus-host disease upon transplantion of the depleted donor cell graft into the recipient.

Second, activation with a DC population, rather than bulk PBMCs, should reduce the non-specific depletion of beneficial donor T cells from an allograft, such as T cells reactive with recipient hematopoietic cells that are useful in mini-transplant procedures, and T cells reactive with leukemic cells. This advantage will particularly be observed using cell populations highly enriched for DCs, and cultured DC populations which contain few hematopoietic stem and progenitor cells.

Third, DCs should be more potent activators of antigen-reactive T cells than PBMCs, facilitating the selective depletion of undesired antigen-reactive populations from a donor graft (e.g. autoantigen-reactive T cells), and the selective isolation of beneficial antigen-reactive cell populations (e.g. tumor-reactive or viral-reactive T cells) for administration to a recipient.

EXAMPLE II

Comparison of the Efficacy of Recipient-Derived DCs and Cultured Macrophages for Activating Reactive Donor T Cells

This example shows that cultured macrophages, like DCs, also potently activate reactive donor T cells. Therefore, a cultured macrophage population can be used as a stimulatory cell population to effectively and specifically deplete (or isolate) reactive T cells from a donor cell population.

A monocyte enriched cell population was prepared as described in Example I, above. The monocyte cell population was either cultured for 7 days in GM-CSF (2500 U/ml) to differentiate monocytes along the macrophage lineage, or cultured for 7 days in GM-CSF (2500 U/ml) and IL4 (1000 U/ml) to differentiate monocytes along the DC lineage.

The cell populations were then examined phenotypically. Cells gated in a fluorescence activated cell sorter as non-lymphocytes, according to their scatter profile (see Figure 1A), were assessed for their CD14 expression. In the macrophage population (GM-CSF cultured monocytes), about 70-90% of the cells were CD14+, most of which were CD86+. In the dendritic cell population (GM-CSF + IL4 cultured monocytes), about 70-90% of the cells were CD14-.

The differentiated populations, as well as the uncultured monocyte population, were then cocultured with a donor PBMC preparation in a mixed lymphocyte reaction, as described in Example I, above. Proliferation of T

cells directly correlates with their activation. Thus, the incorporation of 3H-thymidine into DNA in the MLR, which is a measure of T cell proliferation, was used as a measure of T cell activation.

Figure 5 shows the relative T cell proliferation following coculture of the indicated number of recipient-derived stimulatory cells (either uncultured monocytes, macrophages or DCs) with 1 x 10^5 donor T cells. Under these conditions, uncultured monocytes were unable to activate T cells at the ratios used (circles). Consistent with the results presented in Example I, above, monocyte-derived DCs were able to effectively activate T cells at ratios of from 1:1 DC:T to less than 1:10 DC:T (filled squares). Cultured macrophages were also able to activate T cells, and were most effective at ratios of about 1:5 to 1:8 (open triangles).

Therefore, a cultured macrophage population can be used in therapeutic methods in which it is desired to selectively and effectively deplete or isolate reactive T cells from a donor graft.

All journal article, reference and patent citations provided above, in parentheses or otherwise, whether previously stated or not, are incorporated herein by reference in their entirety.

Although the invention has been described with reference to the examples provided above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the claims.

We claim:

1. A method for depleting reactive T cells from a hematopoietic donor cell population, comprising co-culturing a recipient-derived dendritic cell population or cultured macrophage population with said donor cell population, under conditions wherein said recipient-derived population activates donor T cells in said donor cell population, and killing or removing said activated T cells.

2. The method of claim 1, wherein said hematopoietic donor cell population comprises a leukocyte population derived from bone marrow.

3. The method of claim 2, wherein said leukocyte population comprises a mononuclear cell population.

4. The method of claim 2, wherein said leukocyte population comprises a lymphocyte population.

5. The method of claim 2, wherein said leukocyte population comprises a T lymphocyte population, or subset thereof.

6. The method of claim 2, wherein said hematopoietic donor cell population comprises a blood leukocyte population.

7. The method of claim 6, wherein said blood is peripheral blood.

8. The method of claim 6, wherein said leukocyte population comprises a mononuclear cell population.

9. The method of claim 6, wherein said leukocyte population comprises a lymphocyte population.

10. The method of claim 6, wherein said leukocyte population comprises a T lymphocyte population, or subset thereof.

11. The method of claim 7, wherein peripheral blood leukocyte population is obtained from a donor administered at least one mobilizing agent.

12. The method of claim 7, wherein said mobilizing agent contains an active moiety selected from the group consisting of G-CSF, GM-CSF and FLT3 ligand.

13. The method of claim 1, wherein said recipient-derived dendritic cell population comprises cultured dendritic cells produced by culturing a recipient-derived mononuclear cell population in medium containing at least one factor that promotes the differentiation and/or maturation of dendritic cells.

14. The method of claim 13, wherein said mononuclear cell population comprises an adherent cell population.

15. The method of claim 13, wherein said mononuclear cell population comprises a monocyte cell population.

16. The method of claim 13, wherein said monocyte cell population is produced by a method comprising depleting a human blood mononuclear cell population of B cells and T cells.

17. The method of claim 13, wherein said mononuclear cell population comprises a cirDC population.

18. The method of claim 13, wherein said cultured dendritic cells are characterized as CD14-, CD3-, CD19- and HLA-DR+.

19. The method of claim 13, wherein said mononuclear cell population comprises a CD34+ cell population.

20. The method of claim 19, wherein said dendritic cells are characterized as CD14+ and/or CD1a+ and HLA-DR+.

21. The method of claim 13, wherein said medium is serum-free.

22. The method of claim 13, wherein said culturing is performed in a closed fluid path system.

23. The method of claim 13, wherein at least one of said factors is selected from the group consisting of GM-CSF, IL4, IL7, IL13, TNFα, SCF, FLT3L, CD40L, IFNα, monocyte conditioned medium, LPS, PGE, and calcium ionophore.

24. The method of claim 13, wherein at least one of said factors is GM-CSF.

25. The method of claim 1, wherein said recipient-derived dendritic cell population comprises an uncultured cirDC population.

26. The method of claim 25, wherein said cirDC population is prepared by a method comprising depleting a recipient-derived blood leukocyte population of B cells and T cells.

27. The method of claim 26, further comprising depleting said recipient-derived blood leukocyte population of monocytes, granulocytes or NK cells.

28. The method of claim 26, further comprising depleting said recipient-derived blood leukocyte population of any two cell types selected from the group consisting of monocytes, granulocytes and NK cells.

29. The method of claim 26, further comprising depleting said recipient-derived blood leukocyte population of monocytes, granulocytes and NK cells.

30. The method of claim 25, wherein said recipient-derived blood leukocyte population is obtained from a recipient administered at least one cirDC mobilizing agent.

31. The method of claim 25, wherein said cirDC mobilizing agent contains an active moiety selected from the group consisting of G-CSF, GM-CSF and FLT3 ligand.

32. The method of claim 25, wherein said cirDC are characterized as CD3-, CD19-, CD14-, CD56-, CD15- and HLA-DR+.

33. The method of claim 1, wherein said dendritic cell population comprises at least 2.5% dendritic cells.

34. The method of claim 1, wherein said dendritic cell population comprises at least 20% dendritic cells.

35. The method of claim 1, wherein said dendritic cell population comprises at least 80% dendritic cells.

36. The method of claim 1, wherein said cultured macrophage population comprises macrophages produced by culturing a recipient-derived monocyte population in medium containing at least one factor that promotes the differentiation of macrophages.

37. The method of claim 36, wherein said monocyte cell population is produced by a method comprising depleting a human blood mononuclear cell population of B cells and T cells.

38. The method of claim 36, wherein said factor is GM-CSF or M-CSF.

39. The method of claim 36, wherein said medium is serum-free.

40. The method of claim 36, wherein said culturing is performed in a closed fluid path system.

41. The method of claim 1, wherein said recipient-derived population is proliferation-inhibited.

42. The method of claim 41, wherein proliferation is inhibited by treatment of said cell population with radiation or mitomycin C.

43. The method of claim 1, wherein said co-culturing comprises a period of at least 12 hours.

44. The method of claim 1, wherein said co-culturing comprises a period of at least 48 hours.

45. The method of claim 1, wherein said co-culturing is in serum-free medium.

46. The method of claim 1, wherein said removing step comprises contacting said co-cultured cells with at least one T cell activation marker-specific binding agent, and separating cells bound to said binding agent from cells in said co-cultured population.

47. The method of claim 46, wherein said T cell activation marker-specific binding agent binds a surface activation marker.

48. The method of claim 47, wherein said T cell surface activation marker is a molecule selected from the group consisting of CD25, CD69, CD38, CD71 and CD134.

49. The method of claim 46, wherein said T cell activation marker-specific binding agent binds a secreted activation marker.

50. The method of claim 46, wherein said secreted activation marker is selected from the group consisting of IL2, IL4, IL5, and IFNγ.

51. The method of claim 46, wherein said T cell activation marker-specific binding agent is an antibody.

52. The method of claim 46, wherein said T cell activation marker-specific binding agent is attached to a labeled moiety.

53. The method of claim 52, wherein said labeled moitey is selected from the group consisting of a fluorophore and a magnetic particle.

54. The method of claim 46, further comprising contacting said binding agent with a secondary antibody attached to a labeled moiety.

55. The method of claim 54, wherein said labeled moiety is selected from the group consisting of a fluorophore and a magnetic particle.

56. The method of claim 1, wherein said killing step comprises contacting said co-cultured cells with at least one T cell activation marker-specific binding agent directly or indirectly conjugated to a toxin.

57. The method of claim 56, wherein said toxic T cell activation marker-specific binding agent binds a surface activation marker.

58. The method of claim 57, wherein said surface activation marker is a molecule selected from the group consisting of CD25, CD69, CD38, CD71 and CD134.

59. The method of claim 56, wherein said T cell activation marker-specific binding agent binds a secreted activation marker.

60. The method of claim 59, wherein said secreted activation marker is selected from the group consisting of IL2, IL4, IL5, and IFNγ.

61. The method of claim 56, wherein said T cell activation marker-specific binding agent is an antibody.

62. The method of claim 1, wherein said co-culturing step and said killing or removing steps are performed in a closed fluid path system.

63. The method of claim 1, further comprising isolating said removed reactive cells.

64. The method of claim 1, wherein said recipient derived dendritic cell population is pulsed with at least one antigen prior to co-culturing.

65. The method of claim 64, wherein said antigen is an autoantigen associated with an autoimmune disease.

66. The method of claim 65, wherein said autoantigen is selected from the group consisting of a myelin-derived antigen, desmoglein3, glutamic acid decarboxylase, and acetylcholine receptor.

67. The method of claim 64, wherein said antigen is associated with a disease selected from the group consisting of cancer and an infectious disorder.

68. The method of claim 67, further comprising isolating said removed antigen-reactive cells.

69. The method of claim 1, wherein said hematopoietic donor cell population and said recipient-derived dendritic cell population are derived from the same individual.

70. The method of claim 1, wherein said hematopoietic donor cell population and said recipient-derived dendritic cell population are derived from HLA-matched individuals.

71. The method of claim 1, wherein said hematopoietic donor cell population and said recipient-derived dendritic cell population are derived from haploidentical individuals.

72. The method of claim 1, wherein said hematopoietic donor cell population and said recipient-derived dendritic cell population are derived from HLA-mismatched individuals.

73. A hematopoietic donor cell population depleted of reactive T cells, produced by the method of claim 1.

74. A hematopoietic donor cell population depleted of alloreactive T cells, produced by the method of claim 70.

75. A hematopoietic donor cell population depleted of alloreactive T cells, produced by the method of claim 71.

76. A hematopoietic donor cell population depleted of alloreactive T cells, produced by the method of claim 71.

77. A hematopoietic donor cell population containing donor T cells reactive toward recipient hematopoietic cells and depleted of alloreactive T cells, produced by the method of claim 70.

78. A hematopoietic donor cell population containing donor T cells reactive toward recipient hematopoietic cells and depleted of alloreactive T cells, produced by the method of claim 71.

79. A hematopoietic donor cell population containing donor T cells reactive toward recipient hematopoietic cells and depleted of alloreactive T cells, produced by the method of claim 72.

80. A hematopoietic donor cell population containing donor T cells reactive toward recipient leukemic cells and depleted of alloreactive T cells, produced by the method of claim 70.

81. A hematopoietic donor cell population containing donor T cells reactive toward recipient leukemic cells and depleted of alloreactive T cells, produced by the method of claim 71.

82. A hematopoietic donor cell population containing donor T cells reactive toward recipient leukemic cells and depleted of alloreactive T cells, produced by the method of claim 72.

83. A hematopoietic donor cell population depleted of autoantigen reactive T cells, produced by the method of claim 65.

84. A hematopoietic donor cell population enriched for activated antigen-reactive donor T cells, produced by the method of claim 68.

85. A method for reducing the risk of developing graft-versus-host disease, comprising administering to an HLA-matched recipient the hematopoietic donor cell population of claim 74.

86. A method for reducing the risk of developing graft-versus-host disease, comprising administering to a haploidentical recipient the hematopoietic donor cell population of claim 75.

87. A method for reducing the risk of developing graft-versus-host disease, comprising administering to an HLA-mismatched recipient the hematopoietic donor cell population of claim 76.

88. A method for ablating a recipient immune system, comprising administering to a recipient with a partially ablated immune system the hematopoietic donor cell population of claim 77.

89. A method for ablating a recipient immune system, comprising administering to a recipient with a partially ablated immune system the hematopoietic donor cell population of claim 78.

90. A method for ablating a recipient immune system, comprising administering to a recipient with a partially ablated immune system the hematopoietic donor cell population of claim 79.

91. A method for inducing a graft-versus-leukemic cell reaction, comprising administering to a recipient having leukemia the hematopoietic donor cell population of claim 80.

92. A method for inducing a graft-versus-leukemic cell reaction, comprising administering to a recipient having leukemia the hematopoietic donor cell population of claim 81.

93. A method for inducing a graft-versus-leukemic cell reaction, comprising administering to a recipient having leukemia the hematopoietic donor cell population of claim 82.

94. A method for treating an autoimmune disease, comprising administering to a recipient having an autoimmune disease the hematopoietic donor cell population of claim 76.

95. A method for treating cancer or an infectious disorder, comprising administering to a recipient having cancer or an infectious disease the hematopoietic donor cell population of claim 78.

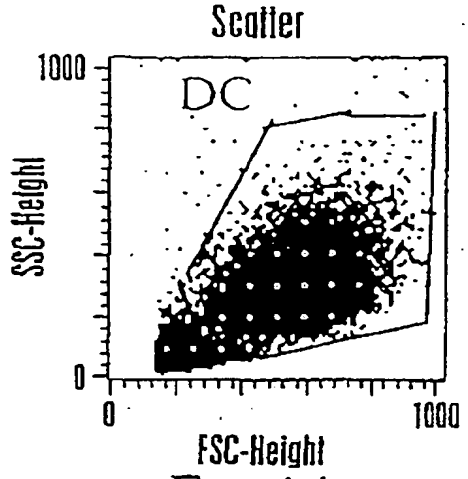

Fig. 1A

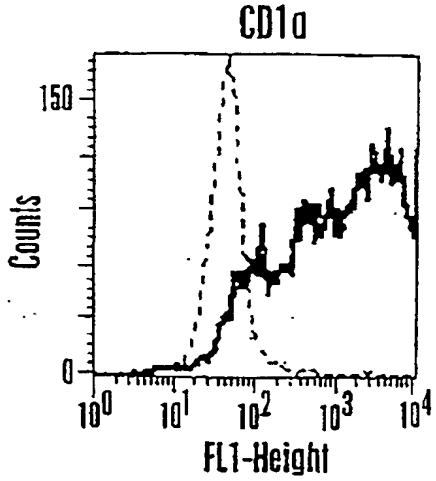

Fig. 1B

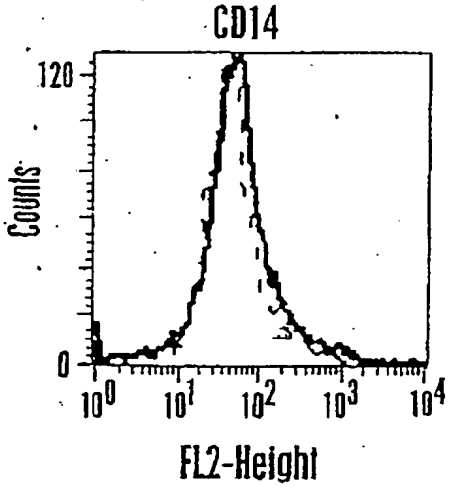

Fig. 1C

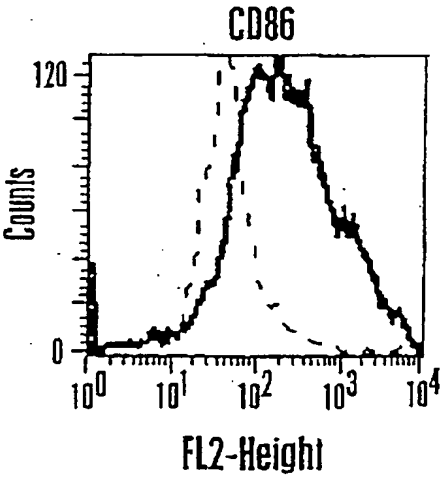

Fig. 1D

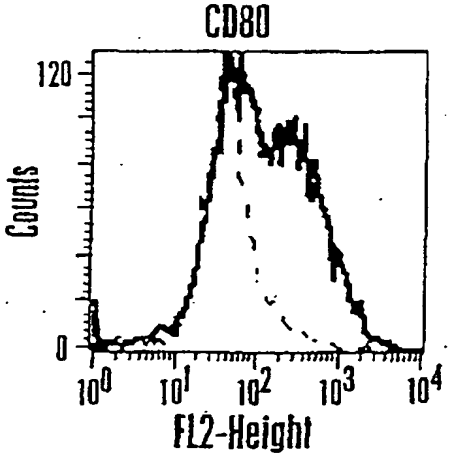

Fig. 1E

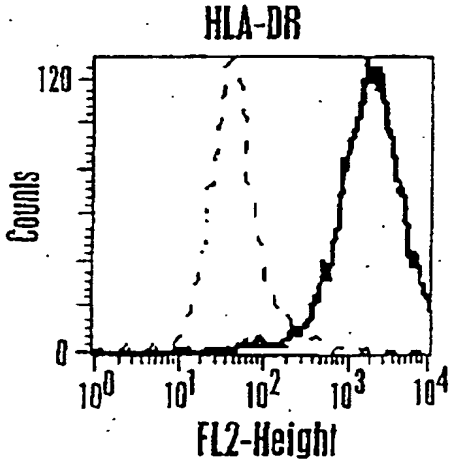

Fig. 1F

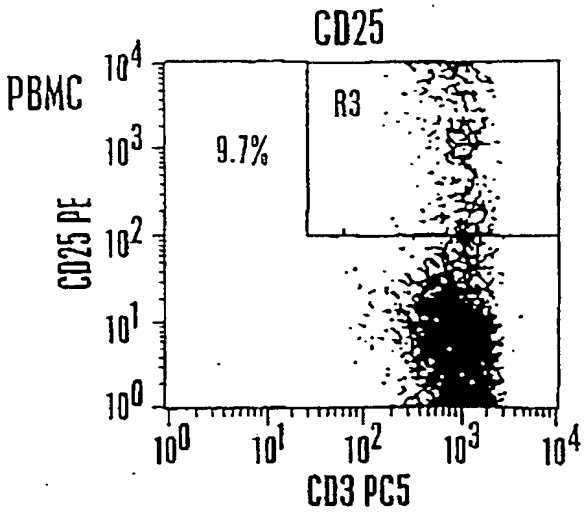

Fig. 2A

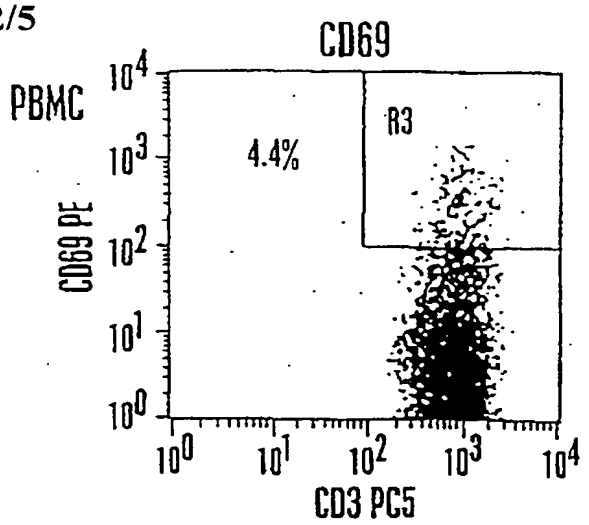

Fig. 2B

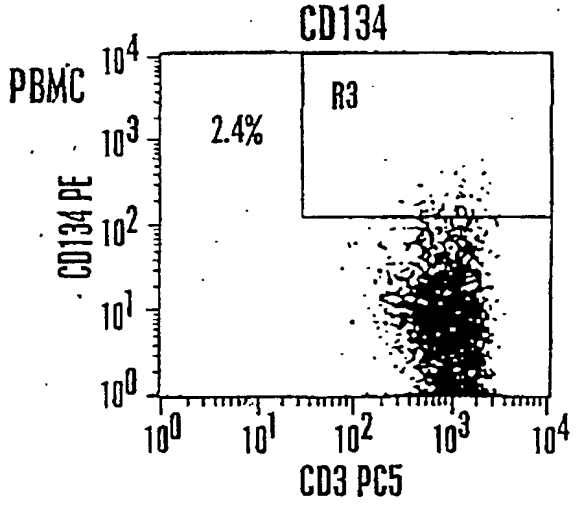

Fig. 2C

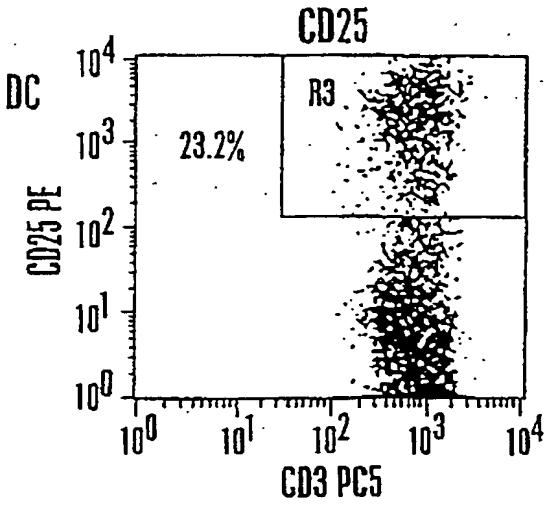

Fig. 2D

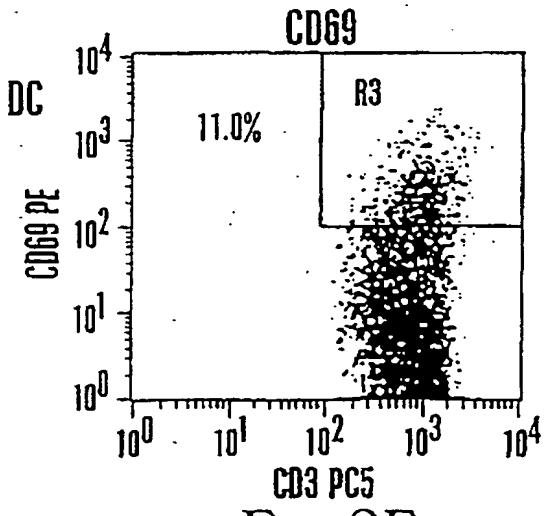

Fig. 2E

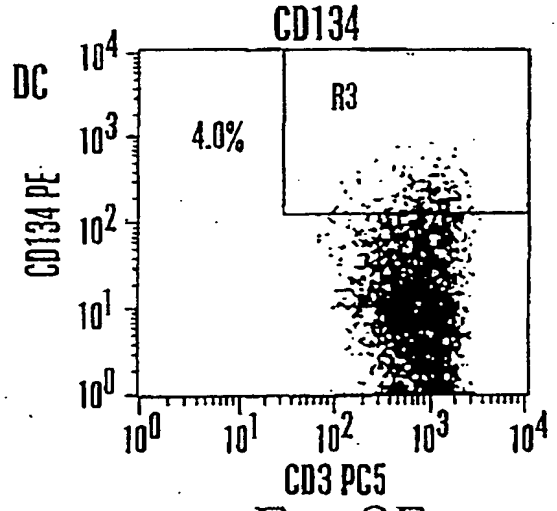

Fig. 2F

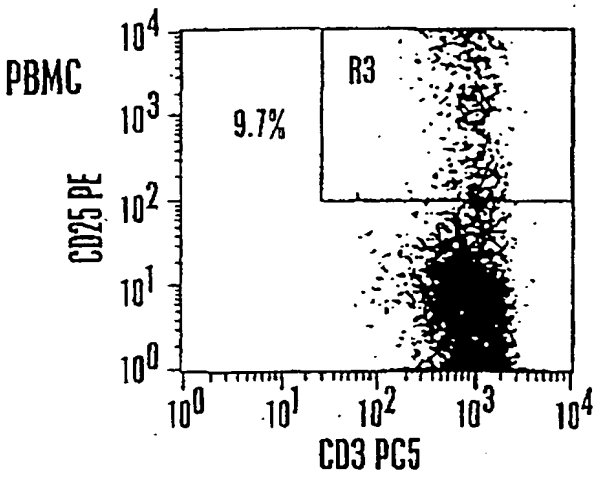

Fig. 3A

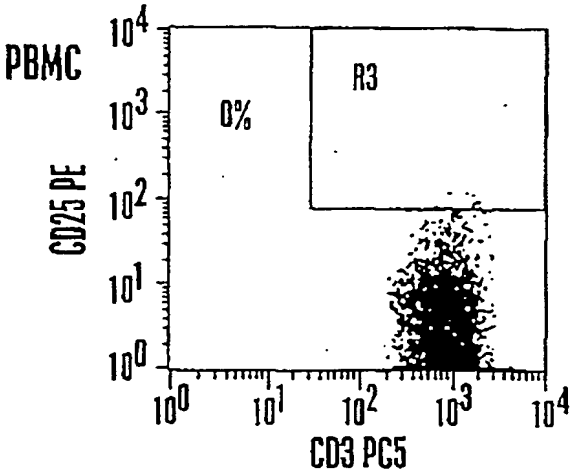

Fig. 3B

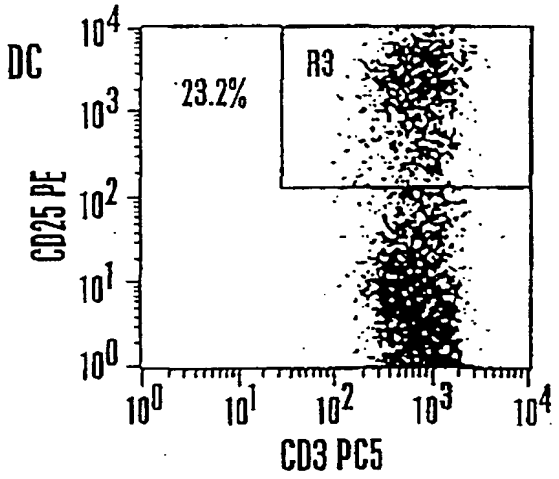

Fig. 3C

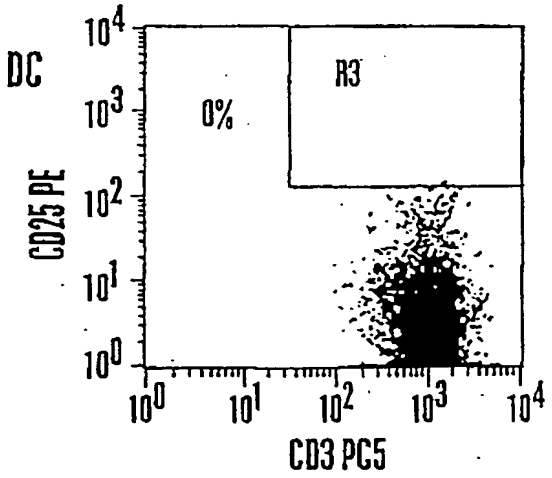

Fig. 3D

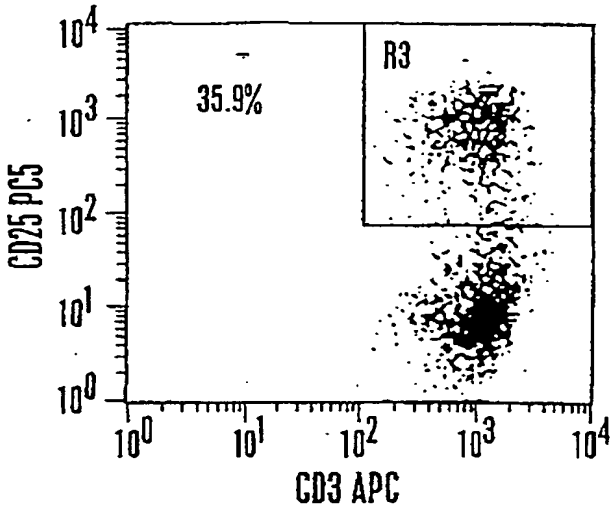

Fig. 4A

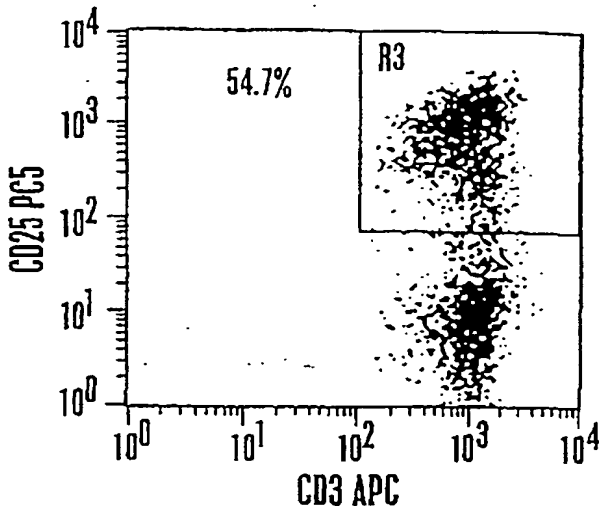

Fig. 4B

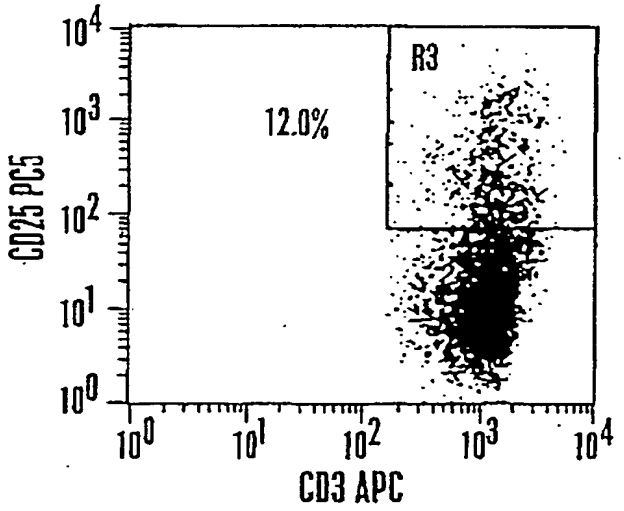

Fig. 4C

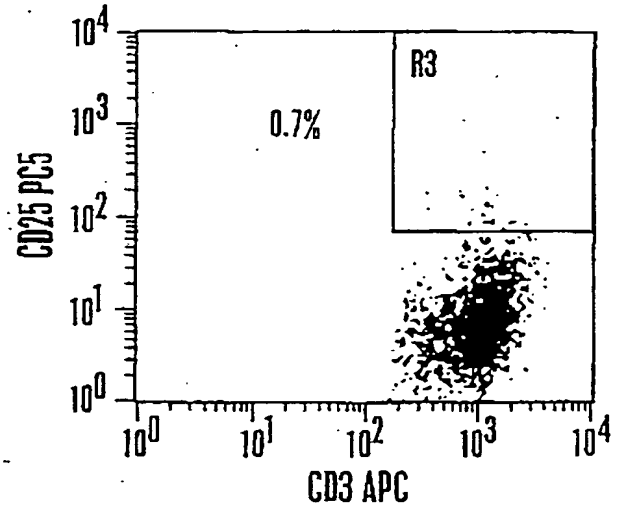

Fig. 4D

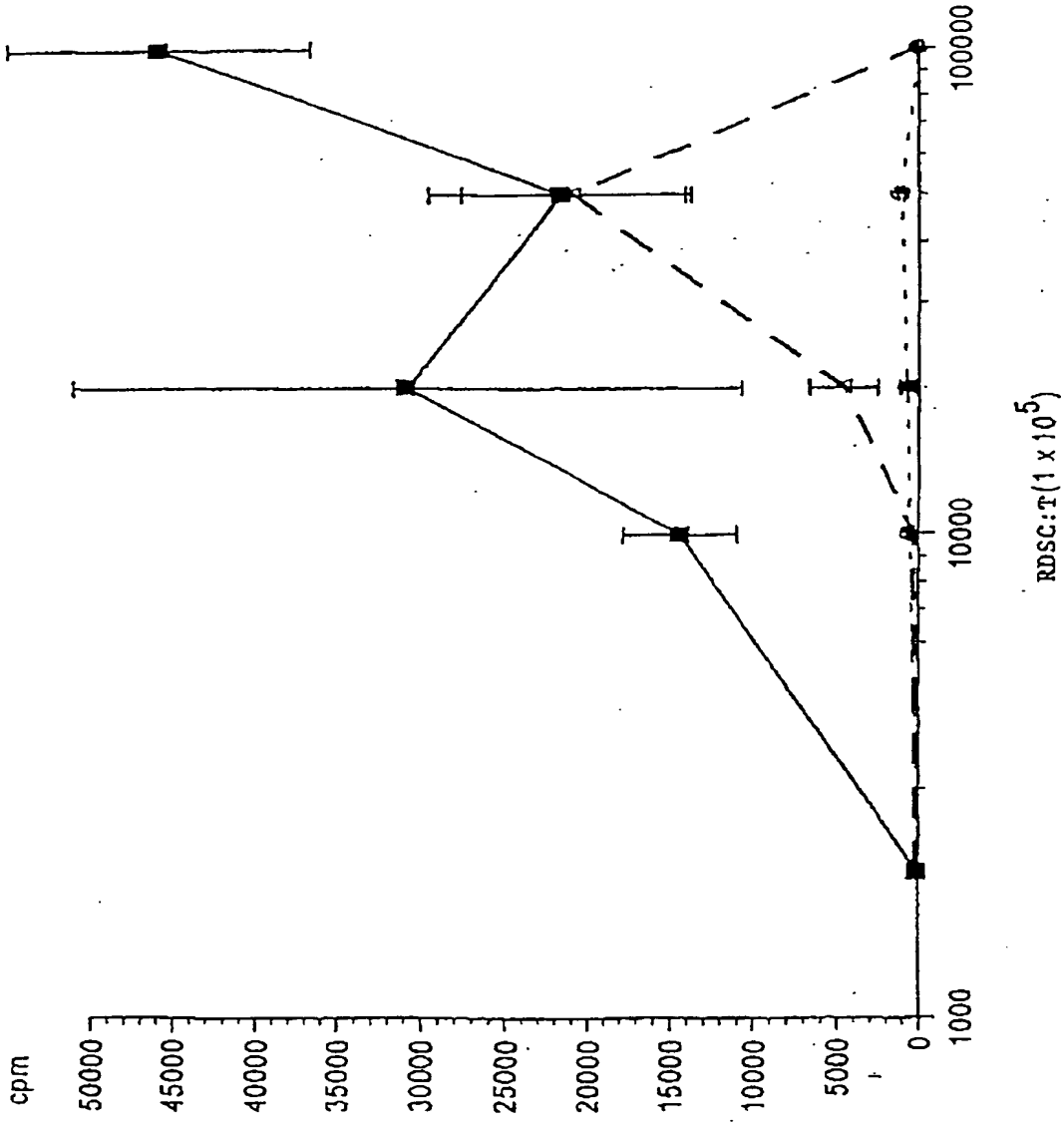
cpm
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
100000
10000
1000
RDSC:T(1 x 10^5)

Fig. 5